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Original Research / Özgün Araştırma

Comparison of the effects of sperm selection methods on assisted reproduction outcomes in male infertility

Erkek infertilitesinde sperm seçim yöntemlerinin yardımcı üreme sonuçları üzerine etkilerinin karşılaştırılması

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Özet

Amaç: Şiddetli veya orta derecede oligoastenospermi olgularında fizyolojik intrasitoplazmik sperm enjeksiyonu (PICSI) ve hipoosmotik şişme testi (HOST) sperm seçim tekniklerini, embriyo gelişimi, implantasyon oranı ve canlı doğum oranları açısından karşılaştırmak.

Gereç ve yöntemler: Medipol Üniversitesi Tüp Bebek Merkezi'ne 2013-2022 yılları arasında başvuran olguların elektronik materyalleri ve dosyaları retrospektif olarak analiz edildi. Ağır veya orta derecede oligoastenospermi tanısı konulan toplam 143 olgu çalışmaya dahil edildi, 80 olguda sperm seçim tekniği olarak PICSI, 63 olguda ise HOST kullanıldı. Dünya Sağlık Örgütü (WHO) tarafından tanımlanan şiddetli veya orta derecede oligoastenospermi vakaları bu çalışmaya dahil edildi. Kadın yaşı, erkek yaşı, infertilite süresi, AMH (anti-Mullerian hormon), vücut kitle indeksi (VKİ), endometrial kalınlık ve önceki deneme sayısı dahil olmak üzere her iki grubun demografik parametreleri ve implantasyon oranı , gebelik kaybı ve canlı doğum oranları gibi transfer sonuçları iki grup arasında karşılaştırıldı.

Bulgular: Kadın yaşı, erkek yaşı, VKİ, AMH, endometrial kalınlık ve önceki deneme sayısı her iki grup arasında benzerdi. Gruplar arasında fertilizasyon (10,2±6,9 vs 9,1±6,4, p=0,345 çok iyi ve iyi kalitede (TQ-GQ) blastokist gelişimi (2,4±2,4 vs 2,6±1,4, p=0,097) ve transfer edilen embriyo sayısı (1,6±0,5 vs 1,8±0,4, p= 0,141) açısından anlamlı fark yoktu.

Abstract

Objective: To compare physiological intracytoplasmic sperm injection (PICSI) and the hypoosmotic swelling test (HOST) sperm selection techniques in terms of embryo development, implantation rate and live birth rates in cases of severe or moderate oligoastheneospermia.

Material and Methods: The electronic material and files of cases admitted to the Medipol University IVF Centre between 2013 and 2022 were analyzed retrospectively. This research included a total of 143 cases with moderate or severe oligoasthenospermia, as a sperm selection technique, PICSI has been used in 80 cases and HOST has been used in 63 cases. Cases of severe or moderate oligoasthenospermia as defined by the World Health Organisation (WHO) are included in this study. The demographic parameters of both groups, including female age, paternal age, duration of infertility, anti-Mullerian hormone (AMH), body mass index (BMI), endometrial thickness, and the number of prior fertility attempts, were analyzed. Implantation rate, pregnancy loss, and rates of live births were compared between two groups.

Results: Female age, paternal age, AMH, endometrial thickness, BMI, and the number of prior fertility attemps were similar between the groups. There were not any significant differences between the groups in terms of fertilization rate (10.2±6.9 vs 9.1±6.4, p=0.345), TQ-GQ blastocyst development (2.4±2.4 vs 2.6±1.4 , p=0.097), and the number of embryos transferred (1.6±0.5 vs 1.8±0.4, p= 0.141). Although the implantation

The study was approved by Ethics Committee of Istanbul Medipol University (Approval Date 2022-12-08 and Protocole Number: 1049). All research was performed in accordance with relevant guidelines/regulations, and informed consent was obtained from all participants.

PICSI ve HOST grupları arasında canlı doğum oranı (p= 0,790) arasında fark olmamasına rağmen HOST grubunda implantasyon oranı PICSI grubuna göre anlamlı olarak daha yüksekti (p=0,043).

Sonuç: İki grup arasında embriyo gelişimi, gebelik kaybı ve canlı doğum oranları açısından fark bulunmadı, ancak HOST yöntemi kullanılan grupta implantasyon oranı daha yüksek idi.

Anahtar Kelimeler: PICSI, HOST, Erkek infertilitesi

rate is significantly higher in the HOST group than PICSI group (p=0.043), the live birth rates were similar.

Conclusion: There were no any differences in embryo development, pregnancy loss, and live birth rates comparing the two methods, however, the HOST group had a higher implantation rate.

Keywords: PICSI, HOST, Male infertility

INTRODUCTION

The aim of in vitro fertilization (IVF) is to achieve a live birth. Approximately 15% of couples who fail to conceive after one year of trying are infertile. 30% to 50% of infertile couples are not able to conceive because of male factor (1). In addition, around 7% of the global male population has been considered infertile (2).

In assisted reproductive technologies (ART), embryo development depends on both egg and sperm quality. There is a correlation between higher chromosomal abnormalities, apoptosis, and DNA damage in males with low sperm count (3, 4). In cases of male infertility, sperm selection methods are essential in order to choose competent sperm. In the female reproductive system, millions of sperm cells compete for fertilization and undergo natural selection, however, in the intracytoplasmic sperm injection (ICSI) process, the embryologist select the spermatozoa solely based on sperm motility and morphology. In terms of competent sperm selection, the separation processes based on motility and morphology, such as swim up (SU) and density gradient centrifugation (DGC), which are the most utilized procedures in ART laboratories today, have not produced satisfying results (5). There are several sperm selection methods that are used to improve IVF outcomes. Physiological ICSI (PICSI) is one of these methods and also based on the ability of the sperm to adhere to Hyaluronic acid (HA) which is present in the cumulus-oocyte complex (COC). The presence of HA in the COC is important for fertilization of the oocyte. Because HA-specific receptors are found in mature sperm. In PICSI technique, sperm that have been washed and centrifuged are put on a PICSI dish with HA-coated spots. After a period of incubation, the spermatozoa surrounded by HA are chosen for ICSI. Some studies have demonstrated that analysis of spermatozoa attached to HA gels revealed normal morphology, low DNA damage, and low chromosomal aneuploidy and PICSI has been shown to improve fertilization rates, embryonic development, and pregnancy outcomes in IVF cycles (6, 7).

The other sperm selection technique is the hypoosmotic swelling test (HOST) that evaluates the integrity of the sperm plasma membrane (8). This test is based on the theory of fluid movement across the cell membrane until equilibrium is established between the inside and outside of the sperm cell under hypo-osmotic conditions. The tails of live spermatozoa swell and bend under these hypoosmotic conditions. The test mainly evaluates the integrity of the tail membrane, however it also reveals that the plasma membrane in the sperm head is intact. Sperm membrane integrity is important for capacitation, sperm metabolism, acrosome reaction, and sperm binding to the oocyte surface. Therefore, HOST is an essential sperm selection technique in male infertility because it assures that sperm cells with better nuclear material will be selected for ICSI (9).

Although many studies have been performed to determine the ideal sperm selection technique, there is still no clear consensus on this issue. Accordingly, this study compared PICSI and HOST sperm selection techniques in patients with oligoasthenoospermia and evaluated the efficiency of these techniques on blastocyst development and clinical pregnancy rates.

MATERIAL AND METHODS

The electronic material and files of cases admitted to the Medipol University IVF Centre between 2013 and 2022 were analyzed retrospectively. The study was compiled according to the principles of the Declaration of Helsinki and was approved by the Medipol University Faculty of Medicine Ethics Committee with protocol number E-10840098-772.02-7443.

This research included a total of 143 cases with moderate or severe oligoasthenospermia, as a sperm selection technique, PICSI has beeen used in 80 cases and HOST has been used in 63 cases. Severe or moderate oligoasthenospermia as defined by the World Health Organisation (WHO) 2021 6th Edition (10) (Table 1). In the study, only ejaculated spermatozoa were used. The cases that not binded to HA on PICSI or a negative HOST test were not included to study. In order to minimize confounding factors, the study included only women with aged 20 to 35. The women with major endocrinological disease (such as congenital adrenal hyperplasia or Cushing's syndrome), endometrial factor, untreated hydrosalpinx, and uterine anomalies verified by hysterosalpingography or hysteroscopy were excluded from the study. The demographic parameters of both groups, including female age, paternal age, duration of infertility, anti-Mullerian hormone (AMH), body mass index (BMI) endometrial thickness, and the number of prior attempts, were analyzed. Implantation rate, pregnancy loss, and rates of live birth were compared between the two groups. On the second day of menstruation, all patients underwent ultrasonographic assessment to rule out ovarian cysts and other pelvic diseases. All research participants underwent a short antagonist protocol. The initial dose of recombinant follicle stimulating hormone (rFSH, Gonal-F®, Merck-Serono, Italy) was determined on BMI, AFC (antral follicle count), AMH, and previous ovarian stimulation responses, if any. Cetrorelix (Cetrotide®, Merck-Serono, Spain) was administered when at least one follicle throughout the cycle reached 12-13 mm in diameter. Every 2-3 days, transvaginal ultrasonography was performed to monitor the development of follicles. Recombinant human chorionic gonadotropin (r-hCG 250 mcg; Ovitrelle®; Serono; Spain) was administered, when three or more follicles reached 18 mm in diameter. Then, 36 hours after the injection of r-hCG, ultrasound-guided transvaginal oocyte retrieval was performed.

Table 1. World Health Organization (WHO) semen analysis 2021 (6th Edition)

Semen volume (mL)	1.4 (1.3-1.5)
Total sperm number (10^6per ejeculate)	16 (15-18)
Total motility (%)	42 (40-43)
Progressive motility (%)	30 (29-31)
Non progressive motility (%)	1(1-1)
Immotile sperm (%)	20 (19-20)
Vitality (%)	54 (50-56)
Normal forms (%)	4 (3.9-4)

On the oocyte pick up (OPU) day, after 3-5 days of ejaculatory abstinence, sperm samples were obtained. For 30 to 60 minutes, the sample of sperm was left at room temperature to liquefy. The analysis of sperm was conducted in accordance with WHO criteria. After performing a swim-up, gradient centrifugation was applied to separate the semen's cellular components. For the selection of mature sperm, sterile PICSI Petri dishes were used (PICSI® Sperm Selector, ORIGIO, Denmark). PICSI dishes are the standard plastic culture dishes pre-prepared with three drops of HA gel. To allow the sperm to bind to the glycosaminoglycan, approximately 2 µl of processed sperm sample was applied to the lateral surfaces of the HA hydrated droplets, and the temperature was retained at 36.8°C for a minimum of 5 minutes. Then, spermatozoa with normal morphology, which were bound to the surface of HA microdots from their heads, were collected using an ICSI injection pipette (ICSI Micropipettes; Humagen Fertility Diagnostics-Origio) and injected into the oocyte. Sperms of cases to be selected by the HOST test were incubated in a hypoosmotic 150 mOsm solution (75 mm) at 37°C for 5-10 minutes following Swim-up and gradient centrifugation (11) spermatozoa with B+ shape in the HOST classification

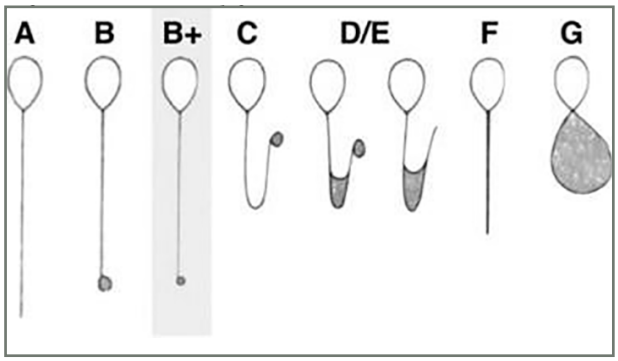

Figure 1. The different categories of HOST defined in the WHO laboratory guidelines for the process and examination of human semen, the addition of type B+

Following ICSI, oocytes were cultured (Life Global®, Belgium) and kept at 37°C and 6% CO2 in a conventional incubator. Additionally, pronucleus (PN) control was conducted on day 2, 18 to 20 hours following ICSI. On day 5, Gardner criteria were applied to grade blastocysts, and for embryo transfer (ET), the TQ and GQ (top quality and good quality) embryos were selected. The ET was conducted using ultrasound guidance. The transfer day and the number of embryos were selected by the doctor based on the patient's and cycle's parameters. Progesterone vaginal gel (Crinone® 8% vaginal gel 90 mg, Merck, UK) or a progesterone vaginal tablet (Lutinus® 100 mg, Ferring, Turkey) were used to support the luteal phase and were given intravaginally twice a day until 12 weeks of pregnancy. Following embryo transfer, serum beta-HCG was tested 9 or 11 days after embryo transfer, depending on the ET day and those with a positive result had pelvic ultrasound three weeks later to confirm intrauterine pregnancy. Detection of the gestational sac by transvaginal ultrasonography was defined as implantation. All cases that did not reach live births were defined as pregnancy loss.

In our study, the rate of live birth was the primary outcome. Fertilization rate, blastocyst development rate, and TQ-GQ blastocyst development rate were the secondary outcomes.

Statistical Analysis

SPSS "SPSS for Windows 22" was used for statistical analysis. $p<0.05$ was considered statistically significant. The distribution of continuous data was checked by the Kolmogrow-Smirnow test. Depending on their distribution, continuous data were compared using Student's t-test. For comparing categorical data, Chi-Square and Fisher Exact test were used. Using Spearman or Pearson correlation analysis, the relationships between continuous data were determined.

RESULTS

Female age, paternal age, AMH, BMI, endometrial thickness, and the number of previous trials were similar between the groups. In total, 143 cases were included in this study. This includes 80 cycles of sperm selection by PICSI and 63 cycles of sperm selection by HOST. The mean female age 30.4 ± 5.2 and 29.9 ± 5.3, p=0.143), male age (34.4 ± 5.4 and 32.9 ± 6.7 p= 0.602), and sperm concentration (7.0 ± 0.9 vs 6.5 ± 1.3, p=0.783) were similar between the groups. Total sperm motility (39.1 ± 14.5 vs 27.9 ± 18.9, p= 0.000) was lower in the HOST group In terms of fertilization rate (10.2 ± 6.9 vs 9.1 ± 6.4, p=0.345), TQ-GQ blastocyst development (2.4 ± 2.4 vs 2.6 ± 1.4, p=0.097), and the number of embryos transferred (1.6 ± 0.5 vs 1.8 ± 0.4, p= 0.141 respectively), no significant differences were determined between the PICSI and HOST groups (Table 2). Although there were not any statistical differences between the PICSI and HOST groups in terms of live birth rate [18/42(%42.8), 12/26(%46), p= 0.790 respectively], the HOST group had a significantly higher implantation rate (p=0.043) (Table 3).

DISCUSSION

In patients with male infertility, sperm selection is essential for embryo development. Sperm selected by ICSI that have normal morphology and motility may have DNA damage, which can have a negative effect on fertilization and IVF outcomes. Consequently, sperm selection techniques that contain sperm functions are

Table 2. Comparison of patient and cycle characteristic

	PICSI n(80)	HOST n(63)	p-value
Male age. (years)	34.4 ± 5.4	32.9±6.7	0.602
Female age. (years)	30.4 ± 5.2	29.9 ± 5.3	0.143
BMI (kg/m2)	26.1 ± 4.3	25.7 ± 4.7	0.583
AMH level. ng/ml	2.9 ± 2.6	3.2 ± 2.8	0.629
Infertility duration. years	5.0 ± 3.8	3.5 ± 2.6	0.009
Previous IVF cycles	0.6 ± 1.1	0.8 ± 1.6	0.370
Total gonadotropin dose (IU/L)	2221.6 ± 702.3	2073.2 ± 443.5	0.144
Endometrial thickness (mm)	10.5 ± 1.9	10.6 ± 1.7	0.673
Estradiol on triggering day. pg/ml	3434.0 ± 1962.0	2852.3 ± 1276.2	0.329
No. of oocyte retrieved	16.2 ± 9.7	14.33 ± 8.4	0.225
No. of mature oocyte	11.5 ± 7.4	10.4 ± 6.8	0.369
No. ICSI fertilizied	10.2 ± 6.9	9.1 ± 6.4	0.345
No.of TQ-GQ embriyos	2.4 ± 2.4	2.6 ± 1.4	0.097
No.of embryo transferred	1.6 ± 0.5	1.8 ± 0.4	0.141
Semen volume (ml)	3.4 ± 1.8	2.5 ± 1.3	0.001
Sperm concentration.10^6/ml	7.0 ± 0.9	6.5 ± 1.3	0.783
Sperm motility	39.1 ± 14.5	27.9 ± 18.9	0.000

Table 3. Comparison of cycle results

	PICSI n=80 n (%)	HOST n=63 n (%)	p-value
No of cancelled embryo transfer	38/80 (%47)	37/63 (%58.7)	
Preimplantation genetic test(PGT)	13/80 (%16)	12/63 (%19)	0.903[2]
Ovarian hyperstimülatıon syndrome(OHSS)	20/80 (%25)	19/63 (%30)	0.741[2]
Cleavage arrest	5/80 (%5)	6/63 (%9)	0.736[1]
No. of embryo transfer cycles	42/80 (%52)	26/63 (%41)	
Implantation rate	22/42 (%52)	20/26 (%76)	0.043[2]
Pregnancy loss rate	4/22 (%18)	8/20 (%40)	0.175[1]
Live birth rate	18/42 (%42.8)	12/26 (%46)	0.790[2]

[1]Fisher-Exact Test. [2]Chi-Square Test. [3]Student t test

crucial, especially in cases of male infertility. Currently, there is no consensus on which method should be used for sperm selection in these cases. Although several studies examine the efficacy of the sperm selection strategies PICSI and HOST, no study has analyzed and compared the outcomes in patients with severe or moderate oligoasthenospermia. All previously reported research compared PICSI to conventional ICSI. Our research is the first to compare PICSI and HOST methods. The primary outcomes of our study, including the rates of live birth, fertilization, and TQ-GQ blastocyst development, did not show differences significantly between the two groups. Although HOST was defined as a simple test to distinguish viable spermatozoa from non-viable spermatozoa at first, later studies have demonstrated that this test has the potential to select the best spermatozoa according to chromatin and membrane integrity, as well as

spermatozoa with a low DNA fragmentation rate (9, 12). In a randomised study comparing the outcomes of ICSI after morphology-based sperm selection with the HOST sperm selection technique, the researchers showed that fertilisation and the rates of pregnancy were significantly higher in the HOST sperm selection group (13). Comparing the sperm of patients whose partners had recurrent early pregnancy loss to the sperm of fertile males, Bhattacharya et al. found that the HOST test scores were lower in the early pregnancy loss group. Therefore, they suggested that the HOST technique for sperm selection may be used to evaluate the association between male factors and recurrent unexplained early pregnancy loss (14). Moskoviz et al. found that the implantation rate in male infertility cases with a normal HOST test was higher, likes the our study results (15).

In our study, the rate of pregnancy loss in cases with severe or moderate oligoastenospermia who underwent sperm selection by PICSI, the miscarriage rate was lower than in the HOST group. Nevertheless, this difference was not statistically significant. A randomised prospective multi-center study comparing ICSI and PICSI did not find any differences in the rates of live birth between the two groups, while PICSI had decreased miscarriage rates (16). Worrilow KC et al. found, in a prospective, multi-center, double-blind, randomized clinical study, that implantation and clinical pregnancy rates were higher in the group using PICSI sperm selection compared to conventional ICSI (17). Although studies comparing PICSI with ICSI in male infertile cases have shown that sperm selection technique with PICSI increases the chance of pregnancy (9, 11) it has been shown that PCSI does not contribute to pregnancy outcomes in cases of unexplained infertility (18).

Our research is the first to compare sperm selection methods, PICSI and HOST. The study is limited by the fact that we were unable to do DNA fragmentation and chromosomal analysis on spermatozoa and that it is a retrospective, single-center study and it has a small sample size. However, we believe that our research might lead to prospective randomized studies.

In conclusion, there is no consensus about the effects of sperm selection methods on the success of in vitro fertilization in male infertility. There were no differences in terms of live birth rate between the two methods. The HOST test that is a simple, cost-effective, rapid, and, non-invasive test, may be preferred in IVF practice.

Conflict of Interest

The authors declare to have no conflicts of interest.

Financial Disclosure

The authors declared that this study has received no financial support.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Ethical Approval

The study was approved by Ethics Committee of Istanbul Medipol University (Approval Date 2022-12-08 and Protocole Number: 1049). The study protocol conformed to the ethical guidelines of the Helsinki Declaration.

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